FEBS Letters 586 (2012) 2778–2779

journal homepage: www.FEBSLetters.org

Review

Antibodies for all: The case for genome-wide affinity reagents

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ARTICLE INFO

Article history:
Received 9 April 2012
Revised 23 May 2012
Accepted 23 May 2012
Available online 1 June 2012

Edited by Marius Sudol, Gianni Cesareni, Giulio Superti-Furga and Wilhelm Just

Keywords:
Domains
Networks
Antibodies
Affinity reagents
Phage display

ABSTRACT

For more than 30 years, the production of research antibodies has been dominated by hybridoma technologies, while modern recombinant technologies have lagged behind. Here I discuss why this situation must change if we are to generate reliable, comprehensive reagent sets on a genome-wide scale, and I describe how a cultural shift in the research community could revolutionize and modernize the affinity reagent field. In turn, such a revolution would pay huge dividends by closing the gap between basic research and therapeutic development, thus enabling the development of myriad new therapies for unmet medical needs.

In most respects, the human genome project has been a resounding success. As a direct result of the initiative, not only do we have access to the complete human genome, as importantly, sequencing technologies have advanced to the point where whole genome sequencing has become a daily affair. Moreover, the astounding advances in DNA sequencing technology have dovetailed with, and often driven, the development of numerous other technologies for the systematic analysis of genomes, transcriptomes and proteomes. Consequently these are best of times for life scientists engaged in basic research. Our basic knowledge of the cell far exceeds what we imagined even a decade ago, and we have the tools to expand this knowledge almost infinitely within the spheres of established technologies.

However, a fundamental tenet of the human genome project was that a complete view of the genome would open up myriad new avenues for therapy. Unfortunately, in this crucial aspect, the promise of the genomics era has not been fulfilled. More than a decade after completion of the genome, the drug development industry has not benefitted greatly from the explosion of basic knowledge, and in fact, the development of novel therapies has declined over this period. Thus, these are the worst of times for researchers engaged in drug discovery. The benefits of genomics and systems biology have not penetrated to the level of drug development and there is no obvious path forward for integrating this wealth of basic knowledge into the practical demands of drug development.

Here, I focus on one problem that has contributed to the vast divide between the promise of genomics and the reality of drug development: the wide gap between the advanced tools for the manipulation of nucleic acids and the relatively primitive tools for manipulation of proteins. Proteins are central to the control of virtually all cellular processes, and virtually all drugs act by modulating the activities of proteins. Thus, it is reasonable to propose that the translation of genomic knowledge to therapeutic development will require a toolkit that enables the manipulation of proteins with the same speed and precision that is commonplace for the manipulation of DNA and RNA. Ideally, this toolkit would be comprehensive, providing us with tools that could track, localize, and modulate any protein in the cell. Such a toolkit would revolutionize our understanding of normal and diseased cellular states, and moreover, these tools could be converted into therapeutics that would act to inhibit or activate proteins in a selective manner to reverse the aberrant protein functions associated with disease.

Given the urgent need for such tools, it is disappointing to contemplate the state of the technology on which our supply of protein affinity reagents depends. The vast majority of affinity reagents are monoclonal antibodies that are still raised using hybridoma technology that has changed little since it was invented more than thirty years ago [1]. The method relies on animal immunization followed by immortalization of individual antibody-producing

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0014-5793
http://dx.doi.org/10.1016/j.febslet.2012.05.044

cells to generate cell lines that produce unique monoclonal antibodies.

Although traditional monoclonal antibodies are used throughout biological research, the hybridoma technology suffers from fundamental drawbacks that have limited affinity reagents from reaching their full potential. First, the process occurs in the uncontrolled serum environment and there is no control over the selection process, which may be suitable for stable antigens, but not for sensitive antigens. In addition, there is no control over which epitopes are targeted, and thus, no guarantee that immunodominant epitopes will be those that are of interest to researchers. Second, the approach provides antibody proteins but not the encoding DNA, and it is not possible to alter or improve the antibody without complicated procedures that convert the molecule to a recombinant form. Third, hybridoma methods involve the use of animals and cumbersome procedures that are inherently unsuited for adaptation to a high-throughput pipeline. Large animal facilities must be maintained to supply the current demands of research groups and the facilities are not scalable without tremendous cost in infrastructure and maintenance. Fourth, natural repertoires are restricted to eliminate antibodies against self, and this makes it difficult to raise effective antibodies against epitopes that are highly conserved across species. The limits of hybridoma technology are particularly acute in the post-genomics era, as the methodology is inherently low throughput and not suited to targeting the tens of thousands of proteins revealed by whole-genome sequencing.

As an alternative to hybridoma technologies, the last decade has seen the emergence of robust molecular display technologies that provide completely controlled approaches to the generation of antibodies and other affinity reagents [2–4]. In phage display, the most popular display technology, libraries of antibody fragments are displayed on phage particles that also encapsulate the encoding DNA [5,6]. Phage pools containing billions of unique antibodies can be used in selections to isolate antibodies that recognize antigens of interest, and the sequence of each antibody can be decoded by sequencing of the linked DNA. In addition, the selections are performed under controlled, in vitro conditions that can be tailored to suit the demands of the antigen.

The combination of display technologies with structure-based design has enabled the development of "synthetic" antibody libraries with man-made antigen-binding sites and libraries built with alternative, non-antibody scaffolds [7,8]. Synthetic repertoires circumvent the need for natural antibodies altogether and possess advantages due to their highly defined nature. Scaffolds can be chosen for high stability and expression, can be modified to suit the demands of particular applications, and can be designed for facile affinity maturation. Unlike natural repertoires, synthetic repertoires are completely naïve and are not biased against self-antigens. These features of phage-displayed synthetic antibody libraries obviate many of the limitations of hybridoma technology, and the system is much more suited to high-throughput applications, since the entire process relies on simple molecular biology techniques.

Not only can recombinant antibody libraries more effectively fill all the applications of hybridoma antibodies, they can also expand the reach of affinity reagents to entirely new areas. For therapeutics, high stability and low immunogenicity are essential, and synthetic antibodies libraries can be designed to directly supply molecules with these characteristics. Also, natural antibodies do not function intracellularly but it is possible to produce synthetic "intrabodies" or alternative scaffolds that are adapted for folding and function inside cells [8]. Recombinant antibodies are also modular, because they can be fused genetically to additional domains to endow additional functions, such as fluorescence to track protein localization and trafficking. Finally, while hybridoma methods have changed little over time, recombinant methods continue to evolve and improve, and almost certainly, new applications for recombinant antibodies will arise as the technologies evolve.

Despite the power of recombinant antibody libraries, hybridoma methods still dominate the field for a number of reasons. The early development of hybridoma methods provided the advantage of familiarity, and for most antibody users, conservatism trumps innovation. In contrast, dissemination of in vitro display technologies was hampered by restrictive intellectual property rights, but most platform patents have either expired or will do so over the next few years. Also, most in vitro technology development efforts have focused on developing more powerful, specialized methods for selections rather than developing simpler, standardized methods that could expand the reach of the technology. As a consequence of these conditions, the research community still has only a vague awareness of the advantages of in vitro antibody technology relative to the more familiar hybridoma technology.

However, the times are changing, and it is becoming clear that complacent reliance on hybridoma methods will not suffice if affinity reagents are to keep pace with advances in genomics. Radical improvements in affinity reagent technologies will be absolutely necessary if we hope to exploit genomic knowledge for therapeutic breakthroughs. Only in vitro methods have the features that will be required to adapt affinity reagent generation to the kinds of high-throughput pipelines that are commonplace in genomics research and other areas of systems biology.

In particular, I believe that the emerging field of high-throughput affinity reagent development is perfectly poised to mesh with the well-established field of protein interactome mapping. The common thread is the understanding that most large proteins can be broken down into smaller domains that can be produced and studied individually. This realization has already enabled the comprehensive analysis of interactomes for many structural domain families. These domains could also be ideal antigens for in vitro high-throughput pipelines for the generation of recombinant antibodies on a genome-wide scale [9]. A pilot study of the SH2 domain family has already proven that protein domains can be used as antigens with remarkable success [10]. By combining domain antigens with synthetic antibody libraries, it should be possible to target the proteome with a comprehensive set of recombinant affinity reagents that would enable the rapid elucidation of protein function. Most importantly, these affinity reagents would also enable the identification of key protein–protein interactions that are involved in disease states, thus providing critical links between our vast stores of genomic data and the urgent need for new therapeutics.

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